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Discussion

Fragments SARS-Cov-2 in aquatic organism represent an additional environmental risk concern: Urgent need for research

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Perspective

Coronavirus Disease-2019 (COVID-19) pandemic, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), an emerging beta-coronavirus that threatens human health and has spread surprisingly fast as a new pathogen (Pan et al., 2022). It is well known that COVID-19 will have global implications for the human population in the long-term

future. Several new mutations of SARS-CoV-2 Binding Domain Spike protein RBD were recently discovered, including Alpha (B.1.1.7); Beta (B.1.351); Range (P.1), and Delta (B.1.617.2) Omicron (B.1.1.529). These variants are rapidly spreading in the UK, South Africa, Brazil, and India and studies suggest that mutations of amino acid RBD K417, E484, L452, T478, and N501 are strongly increasing the affinity of this protein with human angiotensin-converting enzyme 2 (ACE2) (Sanches et al., 2021).

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As far as we know, the classic form of transmission of SARS-CoV-2 is by air and/or via contact with infected people (Meyerowitz et al., 2020; Harrison et al., 2020). However, other forms of transmission of the new coronavirus have been investigated due to the persistence of the virus in the environment for a few hours/day and one of these forms refers to possible contamination/transmission via the fecal-oral or fecal-nasal route (Giacobbo et al., 2021). In spite there is little concrete information on the subject, many researchers have warned about the possibility of infection through direct contact with domestic sewage or contaminated water (Wu et al., 2022; Vo et al., 2022; Baldovin et al., 2021; Sangkham, 2021; Paul et al., 2021; Albastaki et al., 2021; Gonçalves et al., 2021; Westhaus et al., 2021), with the aerosols generated in the systems of wastewater pumping and treatment (Usman et al., 2021; Gormley et al., 2020) and via faulty connections of floor drains interconnected with the main piping of buildings/houses (Shi et al., 2021).

Regardless of whether these studies are still initially to epidemiological conclusions of definitive practical applications, the fact is that the new coronavirus or its fragments have already been identified in different river systems (Guerrero-Latorre et al., 2020; Rimoldi et al., 2020; Mahlknecht et al., 2021). As discussed by Guerrero-Latorre et al. (2020), in countries with a lack of basic sanitation, the spread of SARS-CoV-2 in freshwater environments may be even greater, considering, for example, that in numerous countries less than 30% of the sewage generated is treated before being discharged into the streams (Rodríguez et al., 2020). Consequently, questions arise from this scenario about the extent to which the presence of the new coronavirus (or its fragments) in surface water represents an (eco)toxicological risk for non-target organisms.

Our research group recently reported some effects arising from the exposure of amphibians, fish, and insects to distinct protein fragments of the Spike protein of SARS-CoV-2 (Malafaia et al., 2021; Mendonça-Gomes et al., 2021; Charlie-Silva et al., 2021; Gonçalves et al., 2022; Fernandes et al., 2022). Initially, from a systemic approach (including the synthesis, cleavage, purification, and alignment of three peptide fragments of the SARS-CoV-2 Spike protein, as well as the exposure of neotropical *Physalaemus cuvieri* tadpoles to these fragments) we gathered evidence that confirms the toxicity of the viral constituents in the evaluated animal model. The increase in several biomarkers predictive of oxidative stress and the alteration in acetylcholinesterase (AChE) activity demonstrated that the short exposure (24 h) to these peptides was sufficient to affect the health of tadpoles (Charlie-Silva et al., 2021). In the study by Mendonça-Gomes et al. (2021), we showed for the first time that short-term exposure (48 h) of PSPD-2002 and PSPD-2003 peptides (at 40 µg/L) induced alterations in the locomotor system and in the olfactory behavior of *Culex quinquefasciatus* larvae, which were associated with increased production of reactive oxygen species (ROS) and AChE activity. We also show that exposure to the aforementioned peptide fragments can also alter the behavior of fish (*Poecilia reticulata*), induce redox imbalance, affect the growth and development of animals (Malafaia et al., 2021) and induce genomic instability and DNA damage (Gonçalves et al., 2022). Furthermore, besides representing an important tool to assess the harmful effects of SARS-CoV-2 in the aquatic environment, we present the zebrafish as an animal model for translational COVID-19 research (Fernandes et al., 2022). Therefore, these studies “shed light” on the (eco)toxicological potential of peptide fragments of SARS-CoV-2 in aquatic biota, going beyond the works that have focused on the susceptibility of different mammalian species to viral infection and their roles in the dissemination of COVID-19 (Tiwari et al., 2020; Audino et al., 2021; Delahay et al., 2021).

From this perspective, the study published by Shi et al. (2020), in which the authors describe the transmission of SARS-CoV-2 between cats and ferrets, highlights an important animal model for disease contamination. In addition, Rockx et al. (2020) found that young and old cynomolgus monkeys infected with SARS-CoV-2 also spread the virus. These animal models provide distinct platforms for asking specific questions about SARS-CoV-2 infection, disease induction, and transmission. On the one hand, these studies provide insights into the animal models for SARS-CoV-2 and animal management for COVID-19 control; on the other, they instigate new

investigations on how the permissibility of infection can harm the health of these animals, similarly to what we identified in our studies.

Aquatic organisms, in particular, are not hosts for the SARS-CoV-2, but their particles/peptide fragments can affect the health of these animals, leading to harm to individuals, with the potential to impact their natural populations. Thus, it is urgent that further investigations expand the ecological representation of animal models already studied. Equally essential will be researching how viral particles/fragments access cells. Are amphibians susceptible to absorption through the gills (as tadpoles) and through the skin, as adults? Could such particles/fragments enter the physiological systems of fish, breaking the mucosal barrier of the gills and/or the gastrointestinal system? What cellular mechanisms have the greatest influence on absorption processes, via enterocytes, for example? Are mechanisms or pathways of entry of viral particles/fragments like vertebrates in invertebrates? Is there greater susceptibility to the effects of exposure to these particles/fragments?

Therefore, these are still obscure questions, but they are crucial for us to understand the real magnitude of the indirect effects caused by COVID-19 on aquatic biodiversity. Certainly, this will favor the adoption of measures that can remedy/mitigate the harmful effects on animals, as well as to understand the additional effects of these particles/fragments on the toxicity of so many other pollutants already dispersed in aquatic ecosystems.

CRediT authorship contribution statement

Ives Charlie-Silva: conceived of the presented idea and draft manuscript preparation.

Guilherme Malafaia: conceived of the presented idea and draft manuscript preparation.

All authors reviewed the results and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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